

Complete Genome Sequence of Stenotrophomonas maltophilia Siphophage Salva

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ABSTRACT Stenotrophomonas maltophilia is a Gram-negative pathogen causing severe and often refractory illnesses such as pneumonia and bacteremia. We present the genome of phage Salva, a novel S. maltophilia phage that is not closely related to any phages currently deposited in GenBank. The genome is 60,789 bp, containing 102 putative protein-coding genes.

tenotrophomonas maltophilia is an emerging, Gram-negative, pathogenic bacterium that is often multidrug resistant and is found in environments like animal byproducts, food waste, and other water sources [\(1](#page-1-0)). Infections of S. maltophilia are normally acquired nosocomially, can cause significant life-threatening conditions like pneumonia and bacteremia, and are associated with high mortality rates among certain populations of patients [\(2\)](#page-1-1). Antibiotic-refractory S. maltophilia infections are difficult to treat, so further studies into alternative treatment options, such as phage therapy, are necessary.

Phage Salva was isolated in 2019 from a wastewater sample collected in Cincinnati, Ohio, by enriching the sample using a multidrug-resistant S. maltophilia clinical isolate obtained from human sputum in Decatur, GA, as the host strain aerobically grown at 30°C in nutrient broth (BD), followed by plaque purification ([3](#page-1-2), [4](#page-1-3)). To purify the genomic DNA, the modified Promega Wizard DNA cleanup kit protocol was used ([5\)](#page-1-4). An Illumina TruSeq Nano low-throughput kit was used to prepare DNA libraries, which were sequenced on an Illumina MiSeq instrument with paired-end 300-bp reads using 500-cycle v2 chemistry. A total of 788,720 raw reads were visualized using FastQC v0.11.9 ([www.bioinformatics.babraham.ac.uk/projects/fastqc\)](http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and then manually trimmed using the FastX Toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit). The genome was closed by PCR (with 5'-ATCGCATCTTCGTCCTTTCC-3' and 5'-CTTGTGGCGAAGGTATTCCA-3' as the primer set) and confirmed to be complete by Sanger sequencing. Structural annotations were completed using GLIMMER v3 and MetaGeneAnnotator v1.0 ([6](#page-1-5), [7\)](#page-1-6). tRNAs were detected with ARAGORN v2.36 [\(8](#page-1-7)). To calculate genome-wide sequence similarity, progressiveMauve v2.4 was used ([9\)](#page-1-8). Gene functions were predicted using InterProScan v5.33, BLAST v2.9.0, and TMHMM v2.0 [\(10](#page-1-9)[–](#page-1-10)[12\)](#page-1-11). The sequence similarity search was set at an E value of <0.001 against the NCBI nonredundant and Swiss-Prot/TrEMBL databases. All annotation tools were used with their default settings and hosted (except HHpred) by the Center for Phage Technology (CPT) [\(https://cpt.tamu.edu/galaxy-pub\)](https://cpt.tamu.edu/galaxy-pub) [\(13](#page-1-12)[–](#page-1-13)[15](#page-1-14)).

Phage Salva has a 60,789-bp genome with a GC content of 56.4%, in comparison to its host genome, which has a GC content of 66.7% ([16](#page-1-15)). A long terminal repeat region (3,973 bp) is predicted by PhageTerm [\(17](#page-1-16)) analysis. Phage Salva has one tRNA and 102 putative protein-coding genes, of which 24 have predicted function. Genes that are normally associated with the lysogenic life cycle were not identified in the Salva genome. Salva was predicted to be a siphovirus based on genomic analysis, which was Citation Jefferson B, Yao G, Clark J, Le T, Gonzalez C, Liu M, Burrowes B. 2021. Complete genome sequence of Stenotrophomonas maltophilia siphophage Salva. Microbiol Resour Announc 10:e00083-21. [https://doi.org/10](https://doi.org/10.1128/MRA.00083-21) [.1128/MRA.00083-21.](https://doi.org/10.1128/MRA.00083-21)

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confirmed visually by transmission electron micrography. Salva contains two predicted tape measure chaperone genes, not linked by a programed transcriptional frameshift as is common in many tailed phages ([18](#page-1-17), [19\)](#page-1-18). A typical Gram-negative lysis cassette was identified in the Salva genome, consisting of a class I holin, an antiholin, an endopeptidase endolysin, and a bimolecular spanin complex [\(20](#page-1-19)). Comparative genomic analysis revealed that Salva is not closely related to any known phages or prophage elements currently deposited in GenBank. The only phage that is related to phage Salva is Stenotrophomonas phage vB_SmaS_BUCT548 (GenBank accession number [MN937349.1\)](https://www.ncbi.nlm.nih.gov/nuccore/MN937349.1), which shares 74.3% nucleotide identity over 55% coverage (distributed across the Salva genome), as determined by BLASTn.

Data availability. The Salva genome is deposited under GenBank accession number [MW393850.](https://www.ncbi.nlm.nih.gov/nuccore/MW393850) The associated BioProject, SRA, and BioSample accession numbers are [PRJNA222858](https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA222858), [SRR11558346](https://www.ncbi.nlm.nih.gov/sra/SRR11558346), and [SAMN14609643](https://www.ncbi.nlm.nih.gov/biosample/SAMN14609643), respectively.

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REFERENCES

- 1. Brooke JS. 2012. Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. Clin Microbiol Rev 25:2–41. [https://doi.org/10](https://doi.org/10.1128/CMR.00019-11) [.1128/CMR.00019-11.](https://doi.org/10.1128/CMR.00019-11)
- 2. Moc C. 2020. Current and potential treatment options for Stenotrophomonas maltophilia infections. Contagion Live 5:10–11. [https://cdn.sanity.io/](https://cdn.sanity.io/files/0vv8moc6/contagion/89806526aa730c16865f7a2fa841d15a54338ebc.pdf) fi[les/0vv8moc6/contagion/89806526aa730c16865f7a2fa841d15a54338ebc](https://cdn.sanity.io/files/0vv8moc6/contagion/89806526aa730c16865f7a2fa841d15a54338ebc.pdf) [.pdf.](https://cdn.sanity.io/files/0vv8moc6/contagion/89806526aa730c16865f7a2fa841d15a54338ebc.pdf)
- 3. van Charante F, Holtappels D, Blasdel B, Burrowes B. 2019. Isolation of bacteriophages. In Harper D, Abedon S, Burrowes B, McConville M (ed), Bacteriophages. Springer, Cham, Switzerland. [https://doi.org/10.1007/978](https://doi.org/10.1007/978-3-319-40598-8_14-1) [-3-319-40598-8_14-1](https://doi.org/10.1007/978-3-319-40598-8_14-1).
- 4. Cross T, Schoff C, Chudoff D, Graves L, Broomell H, Terry K, Farina J, Correa A, Shade D, Dunbar D. 2015. An optimized enrichment technique for the isolation of Arthrobacter bacteriophage species from soil sample isolates. J Vis Exp 52781. <https://doi.org/10.3791/52781>.
- 5. Summer EJ. 2009. Preparation of a phage DNA fragment library for whole genome shotgun sequencing. Methods Mol Biol 502:27–46. [https://doi](https://doi.org/10.1007/978-1-60327-565-1_4) [.org/10.1007/978-1-60327-565-1_4.](https://doi.org/10.1007/978-1-60327-565-1_4)
- 6. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27:4636–4641. <https://doi.org/10.1093/nar/27.23.4636>.
- 7. Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res 15:387–396. <https://doi.org/10.1093/dnares/dsn027>.
- 8. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. <https://doi.org/10.1093/nar/gkh152>.
- 9. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. [https://doi.org/10.1371/journal.pone.0011147.](https://doi.org/10.1371/journal.pone.0011147)
- 10. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. [https://doi.org/10.1186/1471-2105-10-421.](https://doi.org/10.1186/1471-2105-10-421)
- 11. Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong S-Y, Lopez R, Hunter S. 2014. InterProScan 5:

genome-scale protein function classification. Bioinformatics 30:1236–1240. [https://doi.org/10.1093/bioinformatics/btu031.](https://doi.org/10.1093/bioinformatics/btu031)

- 12. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580. [https://doi.org/10.1006/jmbi](https://doi.org/10.1006/jmbi.2000.4315) [.2000.4315](https://doi.org/10.1006/jmbi.2000.4315).
- 13. Dunn NA, Unni DR, Diesh C, Munoz-Torres M, Harris NL, Yao E, Rasche H, Holmes IH, Elsik CG, Lewis SE. 2019. Apollo: democratizing genome annotation. PLoS Comput Biol 15:e1006790. [https://doi.org/10.1371/journal](https://doi.org/10.1371/journal.pcbi.1006790) [.pcbi.1006790](https://doi.org/10.1371/journal.pcbi.1006790).
- 14. Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, Taylor J, Nekrutenko A. 2020. Corrigendum: the Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. Nucleic Acids Res 48:8205–8207. [https://doi.org/10.1093/nar/gkaa554.](https://doi.org/10.1093/nar/gkaa554)
- 15. Ramsey J, Rasche H, Maughmer C, Criscione A, Mijalis E, Liu M, Hu JC, Young R, Gill JJ. 2020. Galaxy and Apollo as a biologist-friendly interface for high-quality cooperative phage genome annotation. PLoS Comput Biol 16:e1008214. [https://doi.org/10.1371/journal.pcbi.1008214.](https://doi.org/10.1371/journal.pcbi.1008214)
- 16. Crossman LC, Gould VC, Dow JM, Vernikos GS, Okazaki A, Sebaihia M, Saunders D, Arrowsmith C, Carver T, Peters N, Adlem E, Kerhornou A, Lord A, Murphy L, Seeger K, Squares R, Rutter S, Quail MA, Rajandream M-A, Harris D, Churcher C, Bentley SD, Parkhill J, Thomson NR, Avison MB. 2008. The complete genome, comparative and functional analysis of Stenotrophomonas maltophilia reveals an organism heavily shielded by drug resistance determinants. Genome Biol 9:R74. [https://doi.org/10.1186/gb-2008-9](https://doi.org/10.1186/gb-2008-9-4-r74) [-4-r74.](https://doi.org/10.1186/gb-2008-9-4-r74)
- 17. Garneau JR, Depardieu F, Fortier LC, Bikard D, Monot M. 2017. Phage-Term: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. Sci Rep 7:8292. <https://doi.org/10.1038/s41598-017-07910-5>.
- 18. Xu J, Hendrix RW, Duda RL. 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. Mol Cell 16:11–21. [https://doi](https://doi.org/10.1016/j.molcel.2004.09.006) [.org/10.1016/j.molcel.2004.09.006](https://doi.org/10.1016/j.molcel.2004.09.006).
- 19. Xu J, Hendrix RW, Duda RL. 2014. Chaperone-protein interactions that mediate assembly of the bacteriophage lambda tail to the correct length. J Mol Biol 426:1004–1018. [https://doi.org/10.1016/j.jmb.2013.06.040.](https://doi.org/10.1016/j.jmb.2013.06.040)
- 20. Cahill J, Young R. 2019. Phage lysis: multiple genes for multiple barriers. Adv Virus Res 103:33–70. [https://doi.org/10.1016/bs.aivir.2018.09.003.](https://doi.org/10.1016/bs.aivir.2018.09.003)